Comparison of Phenotype Between Patients With Prader-Willi Syndrome Due to Deletion 15q and Uniparental Disomy 15

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Prader-Willi syndrome (PWS) is a complex multiple anomaly syndrome that has been shown to result from deficient expression of paternal chromosome 15(q11-q13). In most cases, it is caused either by deletion of this region in the paternally inherited chromosome 15 or by maternal uniparental disomy (UPD) of chromosome 15. In order to determine whether there are phenotypic differences between patients whose PWS is caused by these two different mechanisms, 54 affected individuals (37 with deletion, 17 with UPD) were personally examined and studied using molecular techniques. The previously recognized increased maternal age in patients with UPD and increased frequency of hypopigmentation in those with deletion were confirmed. Although the frequency and severity of most other manifestations of PWS did not differ significantly between the two groups, those with UPD were less likely to have a "typical" facial appearance. In addition, this group was less likely to show some of the minor manifestations such as skin picking, skill with jigsaw puzzles, and high pain threshold. Females and those with UPD were also older, on average. Possible mechanisms by which these differences could occur and the implications of these differences for diagnosis are described. Am. J. Med. Genet. 68:433–440, 1997.

KEY WORDS: Prader-Willi syndrome; 15q deletion; uniparental disomy; microdeletion; FISH; methylation analysis

INTRODUCTION

Prader-Willi syndrome (PWS) is a complex multisystem disorder that includes neonatal hypotonia, hypogonadism, developmental disability, childhood-onset obesity, characteristic facial appearance, short stature, and behavior abnormalities [Cassidy, 1984; Holm et al., 1993]. This disorder is caused by the absence of normally active paternally inherited genes on chromosome 15q11q13; the maternally inherited genes are inactive due to imprinting [Nicholls, 1993]. PWS results from deletion of the paternally derived 15q11-q13 in approximately 70% of patients (del 15q) and from maternal uniparental disomy (UPD) for chromosome 15 in most of the remaining patients [Ledbetter et al., 1981; Nicholls et al., 1989; Robinson et al., 1991; Mascari et al., 1992]. A small percentage of patients have a mutation in the putative center involved in the control of imprinting [Reis et al., 1994; Buiting et al., 1995]. In recent years, accurate testing for these molecular abnormalities has been developed and is clinically available. Testing is accomplished through detection of a deletion by fluorescence in situ hybridization (FISH) using commercially available DNA probes [Delach et al., 1994]. UPD can be detected through tracing the inheritance of microsatellite polymorphism alleles within and outside the common deletion region of chromosome 15 from the parents to the affected child by using polymerase chain reaction (PCR) [Mutirangura et al., 1993]. In addition, DNA methylation studies using PW-71 or a methylation-sensitive SNRPN probe identify patients with PWS who have a 15q deletion, UPD 15, or an imprinting mutation [Dittrich et al., 1992; Reis et al., 1994; Gillessen-Kaesbach et al., 1995b].

Investigations of phenotypic differences between those patients whose PWS is due to molecularly detected deletion and those in whom it is due to UPD are just beginning to appear [Gillessen-Kaesbach et al., 1995c; Mitchell et al., 1996]. Before the era of molecular testing, Butler and his coworkers observed that patients with cytogenetic 15q deletions were more likely to be hypopigmented [Wiesner et al., 1987; Butler et al., 1989], and there was a suggestion that those without such deletions had a slightly lower mean IQ [Butler

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et al., 1986]. It has since been learned that hypopigmentation is associated with deletion of a pigmentation gene, *P*, which is not imprinted but is located between the usual deletion breakpoints [Gardner et al., 1992; Rinchik et al., 1993]. Angelman syndrome is a clinically distinct disorder, which can be due to maternal deletion 15q11q13 or to paternal UPD 15, and is presumed to be due to an oppositely imprinted gene (or genes) in the same region of chromosome 15 [Knoll et al., 1989; Malcolm et al., 1991; Nicholls, 1993]. Recent studies have suggested that patients with Angelman syndrome due to UPD have a milder phenotype than those with deletion 15q [Bottani et al., 1994; Gillessen-Kaesbach et al., 1995a].

Therefore we conducted a retrospective review of patients with PWS followed over time by one of us (S.B.C.) in a Prader-Willi Syndrome Management Clinic to compare manifestations in those patients with deletion and those with UPD as determined by molecular testing. Such comparisons could provide valuable information about genetic mechanisms as well as being potentially useful for genetic counseling and management purposes.

METHODS

Subjects and Clinical Findings

Charts of all patients followed in the PWS Management Clinics at the University of Connecticut (1981–1995), University of Arizona (1988–1994), and University Hospitals of Cleveland/Health Hill Hospital (1993–1995) were reviewed in order to identify those who have had confirmed diagnoses based on molecular testing, using the techniques described above. Of these, the 54 patients with completed molecular testing had review of all clinical information present in their clinic charts. Other patients followed in these clinics for many years have not had complete molecular testing either because of lack of available financial reimbursement for these tests, or because of the decision by their parents or guardians not to do testing with no management implications. Others have not been seen since molecular testing became standardized. All patients included in the study satisfied published consensus diagnostic criteria [Holm et al., 1993]. The presence or absence of major, minor, and supportive criteria listed in the published diagnostic criteria were recorded. In addition, maternal age, age at walking, IQ, adult height, and need for psychiatric intervention were also ascertained for each patient. A subjective impression as to whether the face was or was not typical of PWS was made by the examining clinical geneticist (S.B.C.) at the time of prior clinic visits and was abstracted from the record. Photographs taken during clinic visits were reviewed to confirm the impression. In most cases in which the face was judged to be typical, examination documented a narrow bifrontal diameter, almond-shaped palpebral fissures, narrow nasal bridge, and downturned mouth. Information obtained from the records was recorded on a form developed for this purpose, so that there was uniformity of information sought. All examinations were conducted by one individual (S.B.C.), and chart reviews were done by only two of the authors (S.B.C. and M.F.), so that differences in identification of subjective findings were minimized. In some instances, missing historical information was sought by the clinic genetic counselor (S.H.) for completion of the data. This involved asking the patient, parent, or care-giver such questions as whether the patient had exceptional skill with jigsaw puzzles, had sleep disturbances, or had an apparently high pain threshold.

When a specific item of information was not mentioned in the chart or available by inquiry, either positive or negative, or when a measured variable such as IQ was not found in the records, the patient was not included in computations for that item. This explains the differences in denominators seen in data presented in Tables I–V.

Laboratory Methodology

Overall laboratory approach. As described in detail below, each index case was initially studied with high resolution chromosome analysis, and the presence of a deletion was confirmed or refuted utilizing fluorescence in situ hybridization (FISH). The beginning studies involved a combination of probes D15S11 and GABRB3; however, after the commercial release of SNRPN, this probe was utilized alone for the FISH studies. Any questionable cytogenetic or clinical cases were repeated with SNRPN if initial studies with D15S11 and GABRB3 were negative. If FISH studies showed a deletion, no further studies were done. If the molecular cytogenetic analysis did not demonstrate a deletion, UPD and methylation studies were undertaken simultaneously to look for another cause of the disorder. All patients who had molecular studies either had deletion detectable by FISH, or UPD detectable by microsatellite analysis; no patients included in the study had imprinting mutations or other unusual molecular findings.

Cytogenetic analysis. Leukocytes from peripheral blood of the index cases were cultured for high resolution chromosome studies according to a modified method of Yunis [1976]. PHA-stimulated blood leukocytes were cultured for approximately 72 hours in RPMI 1640 with 17% fetal bovine serum. The cultures were synchronized by addition of thymidine for the last 16.5 hours of culture and harvested after the addition of ethidium bromide and colcemid for the last 45 minutes and 25 minutes of culture, respectively. The cells were treated for 8 minutes with 0.075 M KCl and fixed in 3:1 methanol-acetic acid before staining. Chromosomes were GTG banded and 20 chromosomal spreads were examined from each of two cultures.

For FISH analysis, each specimen was studied with either D15S11, SNRPN, or GABRB3 probes localized to the Prader-Willi Syndrome Critical Region, obtained from Oncor Inc. (Gaithersburg, MD). Hybridizations were performed according to the manufacturer's instruction. At least 10 metaphases, and in most cases 20 metaphases, were analyzed for the presence of the D15S11, SNRPN, or GABRB3 on one or both chromosomes 15. Hybridization of the PML probe on 15q22 served as a positive control for the presence of both chromosomes 15 in each metaphase.

DNA extraction. DNA from whole-blood specimens was extracted and purified using a commercial DNA Isolation kit (Puregene, Gentra Systems, Inc., Minneapolis, MN). The DNA pellet was rinsed twice in 70% ice-cold ethanol, briefly dried, and resuspended in 10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0 (final concentration 100–500 ng/µl).

Microsatellite analysis for uniparental disomy. Genomic DNA was isolated from 10 cc of peripheral blood of the index cases and, whenever possible, both parents. UPD was determined using highly polymorphic microsatellite markers both within the Prader-Willi Syndrome Critical Region (15q11-q13) and outside this region and analyzed by PCR using standard techniques. Primer sequences for D15S11 and GABRA5 (Operon Technologies Inc., Alameda, CA) and D15S18, D15S128, D15S113, and D15S165 (Research Genetics, Inc., Huntsville, AL) were used in all cases, and additional primer sets were used if these were not informative. One strand of each primer was end-labeled for 1 hour at 37°C in a 10-µl volume consisting of 1.2 µM primer, 25 µCi [gamma-32P] ATP at 3000 Ci/mmol (NEN Research Products, Boston, MA), 50 mmol Tris-HCl, pH 7.5, 10 mmol $MgCl_2$, 5 mmol dithiothreitol, and 5 units T4-Polynucleotide kinase (Boehringer Mannheim). PCR amplification was performed in an MJ Research PTC-200 thermocycler with 30 cycles of 45 seconds at 95°C denaturation, 45 seconds at 56°C annealing, and 1 minute at 72°C extension in a final volume of 25 µl. Each reaction consisted of approximately 300–500 ng genomic DNA, 200 µM dNTPs (Boehringer Mannheim), 0.4 µM unlabeled primer, 0.06 µM labeled primer, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.32 unit *Thermus aquaticus* DNA Polymerase (Life Technologies, Gaithersburg, MD). After amplification, the reaction was mixed with an equal volume of formamide loading buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol), denatured at 95°C for 5 minutes, and placed on ice. Then 4 µl of each sample was directly loaded on to a 0.4-mm-thick 6% "Long Ranger"-modified acrylamide gel (FMC Bioproducts, Rockland, ME), electrophoresed for 3 hours at 50 W, followed by exposure to Fuji RX x-ray film for 2–4 hours at room temperature.

Southern blot hybridizations: methylation analysis. Restriction endonuclease digestion and electrophoresis was done using standard techniques: 10 µg of DNA was doubly digested with 50 U Not1 and 50 U Xba1 (Promega)—according to manufacturer's instructions—overnight at 37°C. Electrophoresis on 0.9% agarose gels was followed by overnight capillary transfer of DNA to nylon membranes (Hybond-N+, Amersham) in 10 × SSC. DNA was crosslinked to the membrane with UV Stratalinker 1800 (Stratagene) and prehybridized for 2–4 hours at 48°C in 50% formamide, 6 × SSC, 0.5% SDS, 5 × Denhardt's solution, and 0.5% nonfat dry milk. DNA probe labeling with [A-32 P]-dCTP (3000 Ci/mmol, New England Nuclear) was done using the High Prime method (Boehringer Mannheim) to a specific activity greater than 1.0×10^9 cpm/µg DNA. Hybridizations were performed overnight at 48°C without replacing hybridization solution. Membranes were washed 15 minutes at room temperature, followed by two washes at 58°C for 15 minutes with 0.1 × SSC, 0.1% SDS. The SNRPN DNA probe was utilized for all the methylation studies.

Statistical Analysis

Statistical assessment of differences in continuous measures between the deletion and disomy groups (or between males and females) was carried out with Student t tests as well as nonparametric median tests. Tests of categorical measures were carried out using Fisher's exact test [Dixon and Massey, 1969]. Two-tailed p values were computed. Since data associated with each outcome or phenotype was not available on each person, as explained in the text, multivariate tests could not be performed. In addition, p values were not corrected for multiple comparisons. We chose not to correct for multiple comparisons because of the exploratory nature of our study and because we believe that many of the features exhibited by patients with deletion vs. UPD are associated or correlated, although this hypothesis requires confirmation with complete data on a large number of subjects.

RESULTS

Of the approximately 200 patients followed in one of the three PWS clinics for whom records were available,

TABLE I. Age at Walking, and Height in Patients With Deletion and UPD

Characteristic	Deletion 15q [range] (*N*)	Disomy 15 [range] (*N*)	*P* value	
			t test[a]	Non-Para[b]
Mean IQ	69.8 [45–82] (12)	63.3 [48–70] (14)	0.315	0.624
Age at walking	27 mo [15–48] (15)	24 mo [17–36] (13)	0.442	0.522
Adult height				
Male	158.6 cm [152–164] (7)	155 cm [145–163] (3)	0.377	0.500
Female	144.6 cm [137–157] (9)	149.4 cm [139–159] (6)	0.180	0.084

[a] Student t test.
[b] Nonparametric analysis.

TABLE II. Manifestations of PWS for Which There Were no Significant Differences Between Patients With Deletion 15q and Maternal UPD

Characteristic	Deletion 15q No.+/total (%)	Disomy 15 No.+/total (%)	*P* value[a]
Severe neonatal hypotonia	26/27 (96)	16/16 (100)	0.569
Need for tube feedings	11/19 (58)	7/14 (50)	0.460
Cryptorchidism[b]	18/18 (100)	8/8 (100)	—
Hypoplastic genitalia[c]	23/26 (88.5)	11/11 (100)	0.335
Small hands/feet[d] (>10 yr)	17/20 (85)	10/11 (91)	0.552
Scoliosis (>15 yr)	13/16 (81)	7/10 (70)	0.420
Dental abnormalities[e]	14/20 (70)	6/11 (55)	0.225
Thick viscous saliva	28/28 (100)	15/16 (94)	0.478
Psychiatric intervention	12/19 (63)	10/17 (59)	0.450
Temper tantrums	23/26 (88.5)	12/16 (75)	0.236
Hyperphagia	21/29 (72)	12/17 (71)	0.460
Decreased vomiting	9/14 (64)	6/12 (50)	0.368
Sleep disturbance	12/25 (48)	7/13 (54)	0.500

[a] Test statistic computed to get *P* values was Fisher's exact test.
[b] Unilateral or bilateral.
[c] Males: hypoplastic scrotum with or without small penis; females:hypoplastic labia minora and clitoris.
[d] Hand length <10th centile; foot length <3rd centile.
[e] Multiple caries, tooth grinding, gum disease, severe malocclusion.

appropriate complete molecular testing had been done on 54. Of these, 37 patients had 15q deletion (70%) and 17 had maternal UPD (30%). These proportions are similar to those found in other studies [Robinson et al., 1991; Mascari et al., 1992; Gillessen-Kaesbach et al., 1995b, 1995c]. There were 24 females (16 with deletion, 8 UPD) and 30 males (21 with deletion, 9 UPD). Age range at time of most recent evaluation was 2 months to 57 years (deletion patients: 2 months to 57 years, mean 16 years; UPD patients: 3.5 years to 40 years, mean 17.6 years). The average age of females (22 years, range: 14 months to 39 years) was greater than that of males (13 years, range: 2 months to 36 years) (*p* value: 0.0381), and all 9 individuals below age 3 years had deletion, all but two of them being male.

The manifestations sought and the results identified in the patient records are shown in Tables I to III. Those findings for which there were no significant differences between the two groups are shown in Tables I and II; those for which there was a significant difference are listed in Table III. Examples of facial phenotypes judged as typical and atypical are shown in Figure 1.

No significant differences between the two groups were found in the mean IQ, age at walking, or the adult height of males or females, and the numbers did not differ from those to be found in the literature [Butler et al, 1986; Cassidy, 1984; Robinson et al., 1991; Holm et al., 1993]. Similarly, there were no significant differences between the frequency in the two groups of most other clinical findings, including neonatal hypotonia, need for gavage feeding, cryptorchidism, hypoplastic genitalia, small hands and feet, scoliosis, dental anomalies, viscous saliva, behavioral disturbances, hyperphagia, decreased vomiting, or sleep disorder.

A few statistically significant differences were found, however, between the two groups. These included the frequency of "typical" facial appearance, unusual skill with jigsaw puzzles, skin picking, and high pain threshold, all of which were higher in the deletion group. The higher frequency of hypopigmentation among those with deletion was also confirmed. All of these are subjective in reporting or interpretation. In addition, there was a trend for a higher frequency of articulation abnormalities among those with deletion, though it barely missed being statistically significantly different ($P = 0.0574$).

The proportion of patients with atypical findings was not greater in either group (Table IV).

Maternal age was ascertained in the two groups, and was advanced among the patients with UPD but not with deletion, as shown in Table V.

TABLE III. Significantly Different Manifestations of PWS in Patients With Deletion 15q and Maternal UPD

Characteristics	Deletion 15q No.+/total (%)	Disomy 15 No.+/total (%)	*P* value
Typical facies	33/36 (92)	10/17 (59)	0.0036
Skill with jigsaw puzzles	13/13 (100)	1/4 (25)	0.0059
Skin picking	21/27 (78)	7/17 (41)	0.0165
High pain threshold	10/10 (100)	3/6 (50)	0.0357
Hypopigmentation	16/33 (48.5)	2/13 (15)	0.0380
Articulation abnormality	22/23 (95.7)	10/14 (71.4)	0.0574

TABLE IV. Uncommon Findings Among Studied Patients

	Deletion 15q $N = 37$	Disomy 15 $N = 17$
Seizures*	5	1
Eye anomalies[a]	2	1
Hearing loss	2	—
Ataxia[b]	1	—
Pervasive developmental disorder	—	1
Breasts cysts	3[c]	—
Asthma	2	2
Congenital heart defect	2	—
Palatal anomaly[d]	2	1
Reflux/rumination	1	2
Gastrointestinal disorder[e]	—	3
Inguinal hernia	2	3
Urinary tract infections	1	2
Duplicated collecting system	1	—
Hypospadias	—	1
Congenital foot deformity	1	2
Hip dysplasia	—	1
High lactate	—	1
XXY (Klinefelter syndrome)	—	1

* $P < 0.36$.
[a] One each: optic atrophy, nystagmus, retinopathy.
[b] Onset in 40s.
[c] One of these was male.
[d] Pharyngeal flap procedure (2), bifid uvula.
[e] One each: spastic colon, Crohn disease, hemorrhoids/rectal bleeding.

DISCUSSION

Although this study is small and retrospective, it suggests that, unlike that proposed for Angelman syndrome [Bottani et al., 1994, Gillessen-Kaesbach et al., 1995a], PWS due to UPD may not be milder than that due to deletion in ways that significantly affect prognosis or management. A similar conclusion was drawn by two other recent studies [Gillessen-Kaesbach et al., 1995c; Mitchell et al., 1996]. Our study confirms the known difference in frequency of hypopigmentation, which is associated with the presence of the *P* locus, representing a gene for type II oculocutaneous albinism, at the distal end of the usual deletion region [Wiesner et al., 1987; Butler et al., 1989; Gardner et al., 1992; Rinchik et al., 1993]. This gene is thought not to be imprinted [Rinchik et al., 1993]. Presumably, the allele on the normal member of the chromosome 15 pair in patients with deletion codes for a protein associated with fair coloring in some, but not all, patients, and the differences in pigmentation of deletion patients results from allelic variability at the *P* locus. Our study also confirms the previously observed finding that maternal age is advanced among patients with UPD, but not among those with deletion [Robinson et al., 1991; Gillessen-Kaesbach et al., 1995b, 1995c]. This has been related to the mechanism of meiotic nondisjunction as a cause of UPD, and its relationship to advanced maternal age [Cassidy et al., 1992; Cassidy, 1995; Robinson et al., 1993].

TABLE V. Maternal Age of Patients With Deletion and Disomy

	Mean maternal age*	N
Deletion	26.8	23
UPD	34.4	12

* $P < 0.001$.

A few phenotypic differences were noted between the two groups; most important, from the diagnostic and clinical perspective, is the finding that the facial phenotype is less often typical in patients with UPD. The assessment of whether the facial phenotype is or is not typical is subjective, based on observer judgment. In this study, there was only one observer, eliminating the possibility of interobserver differences in the perception of "typical." In addition, the observer (S.B.C.) has longstanding experience with ongoing management of over 200 patients with PWS, likely increasing the validity of the assessment. This difference in the presence of typical facial appearance was not noted in four previous studies [Robinson et al., 1991; Gillessen-Kaesbach et al., 1995a, 1995c; Mitchell et al., 1996]. However, each of those studies had several observers, and in some of them blood samples and clinical information were sent in by multiple clinicians, few of whom were highly experienced with PWS [e.g., Gillessen-Kaesbach et al., 1995c]. However, in Robinson et al. [1991], the two clinical observers were very experienced with PWS and identified a typical facial appearance in all methylation-positive patients (and 1 of 6 methylation-negative patients). The reason that their assessment of facial appearance differs from ours is unclear. Possible bias was introduced in our study by familiarity with the patients' molecular status in a few cases; however, the assessment of whether the patient had typical facial appearance was extracted from prior clinic records in most cases, most of which were recorded prior to the availability of molecular testing. Clinical diagnosis of PWS is not always easy, and many patients diagnosed by referring physicians (including some clinical geneticists) and referred to our PWS clinics do not have PWS based on clinical or molecular criteria. Clearly, the identification of "typical face" is somewhat subjective, as is true for most syndromes (e.g., Williams syndrome, Angelman syndrome). However, in our assessment, patients whose facial appearance is less characteristic, often due to a rounder face with broader forehead and flatter or broader nasal bridge, are more difficult to diagnose with certainty on clinical grounds alone. Indeed, none of the "typical" manifestations of PWS are routinely measured, and most are not measurable.

If this observation of less characteristic facial phenotype in PWS due to UPD is confirmed, it might explain why, in our study, the lower limit of the age range for patients with deletion was 2 months, whereas that for UPD was 3.5 years. Regrettably, the age at diagnosis was not recorded in many patients, so that differences could not be ascertained from this study. This would be a useful figure to obtain in subsequent studies. Such a finding could lead to ascertainment bias in referral of UPD cases, if they are less typical. Also, later referral could lead to some distortion of results.

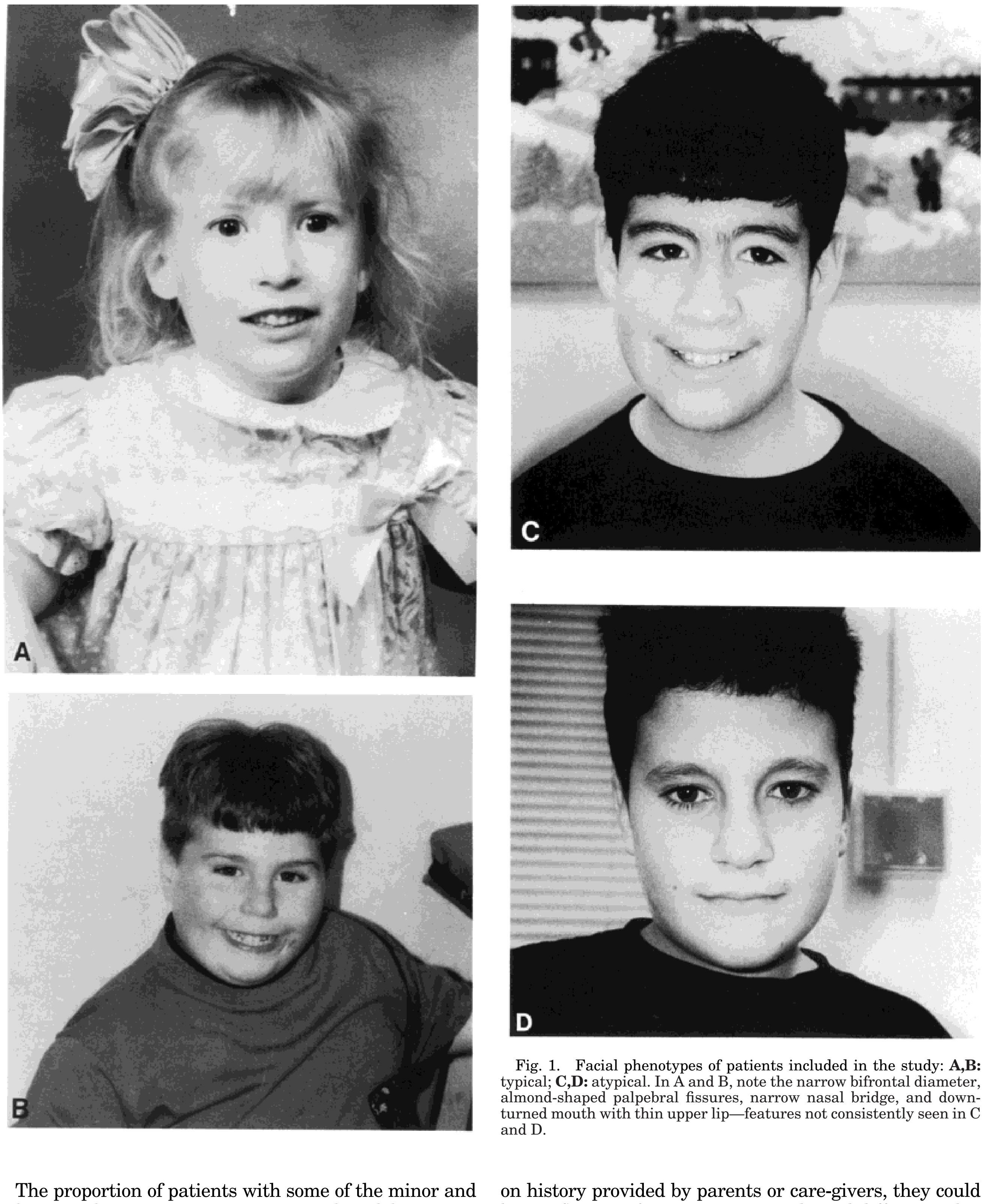

Fig. 1. Facial phenotypes of patients included in the study: **A,B:** typical; **C,D:** atypical. In A and B, note the narrow bifrontal diameter, almond-shaped palpebral fissures, narrow nasal bridge, and down-turned mouth with thin upper lip—features not consistently seen in C and D.

The proportion of patients with some of the minor and subjective characteristics was greater when deletion was present. Patients in the deletion group were more likely than patients with UPD to have skill with jigsaw puzzles, to skin pick, and to have a high pain threshold. While reporting could influence these measures, since they depend on history provided by parents or care-givers, they could be a reflection of cognitive or neurological differences between the two groups of patients. There was also a trend for a higher frequency of articulation abnormality among patients with deletion, again a possible reflection of neurologic or perhaps structural nasopharyngeal differences.

While the proportion of males and females with each type of molecular finding did not differ, the age of males and females differed significantly. In particular, there were no females with UPD below age 8 years. The diagnosis of PWS may be more difficult in females because of the more evident genital hypoplasia in infant males. This would be expected to lead to significant delay in diagnosis in females, particularly for those with UPD, who might also have a less typical facial phenotype than those with deletion. Although their data were obtained and analyzed differently, Gillessen-Kaesbach and colleagues [1995] and Mitchell and colleagues [1996] also noted differences between patients with deletion and UPD when they were analyzed separately by sex. Gillessen-Kaesbach and colleagues [1995] found that males and females with deletion had significantly lower birth weight than nondeletion patients. Mitchell and coworkers [1996] found that males with UPD were significantly shorter at birth than males with deletion, and that females with UPD required a shorter course of gavage feeding and had a later onset of hyperphagia. They also found that fewer females than males in both molecular classes had weight greater than the 95th centile. The data from our patients were insufficient to make these comparisons possible. However, while some males in both groups had many months of gavage feeding, no female in either group had gavage feeding for more than 2 months, and only one of the seven disomic patients with recorded need for gavage feeding was female. This may reflect a lesser degree of hypotonia among females, particularly those with UPD. However, this information is difficult to assess objectively based on description or history only. Again, if hypotonia is less severe among females and/or those with UPD, it could contribute further to delayed diagnosis. Data on weight were not analyzed in our study, since many of the patients have been followed in our management clinic for years, and diet and exercise are an integral component of management.

There was no striking increase in the frequency of rare findings in patients with UPD compared to those with deletion. Nonetheless, the possibility exists that rare recessive mutations will be duplicated through maternal UPD if there is isodisomy, leading to expression of the recessive phenotype. However, most cases of UPD demonstrate recombination, so that only a small part of chromosome 15 will actually show isodisomy [Robinson et al., 1993]. Since all deletion testing was done with FISH probes, it is not possible to determine from our study whether some of the deletion patients with rare findings had deletions larger than the usual deletion, which would encompass loci for genes that predispose to some of the rare malformations. Seizures, the most frequent rare finding, were not statistically different between the two groups. They may occur at increased frequency in patients with PWS as a whole—particularly in those with deletion—suggesting the presence of a gene that contributes to seizures in the PWS/AS region.

Do the relatively minor phenotypic differences between the two groups indicate that there are subtle genetic differences between them? The present study suggests that the answer is affirmative, although none of these differences appears to have a major influence on the natural history, with the possible exception of age at diagnosis. Current thinking suggests that the expression of those paternally active genes that normally prevent the manifestations of PWS is absent in all cases, either because of deletion of the relevant normally active paternally contributed genes or because of the presence of maternal UPD with the resultant lack of expression of those same genes. Whether the phenotypic consequences of these two very different genetic mechanisms are identical or nearly identical is unknown, though this study and others to date suggest they are very similar. However, could there be a nonimprinted gene (or genes) within the usual deletion region that contributes to the "typical" facial phenotype (presumably "typical," since there are more patients with the deletion than with UPD)? This gene would be present and presumably active in patients with UPD. There is some indirect evidence for this possibility [Gillessen-Kaesbach et al., 1995c; LaSalle and Lalande, 1995]. If this is the case, the same phenomenon would be expected in patients with both PWS and AS due to UPD. An alternative possibility is that imprinting may be a "leaky" process, and those with UPD may have a low level of expression from both chromosomes 15, a level that would be higher than in deletion cases, where there is only one dose of "leaky" expression [Bottani et al., 1994], or that occurs only when two chromosomes 15 are present [LaSalle and Lalande, 1995]. It is unknown whether "leakiness" differs in males and females. Yet a further possibility is that one or both of the breakpoints for the deletion interrupt a gene whose consequence is an increase in phenotypic effect. These mechanisms have been proposed as causes of the reported milder phenotype in AS patients with UPD [Bottani et al., 1994; Gillessen-Kaesbach et al., 1995a].

In light of possible subtle phenotypic differences between patients with PWS on the basis of deletion 15q versus UPD 15, should clinicians or clinical laboratory geneticists change their behavior with regard to diagnosis of PWS? The data are insufficient for clear recommendations, but it would appear that the threshold for clinical suspicion of PWS should be lower than it is currently, particularly for females. A blinded study comparing cases of PWS with deletion and with UPD, evaluated by a single clinical geneticist, would be of value, as would a study designed to address differences in age at diagnosis between these two groups. Any discrepancies would be useful both in clinical diagnosis of patients and in understanding the molecular pathogenesis of PWS.

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